1. PURPOSE

1.1. The purpose of this procedure is to describe the use and maintenance of the Nexcelom Cellometer Auto 2000.

2. SCOPE

2.1. This procedure applies to the Human Papillomavirus (HPV) Serology Laboratory located at the Advanced Technology Research Facility (ATRF), room C2007.

3. REFERENCES

3.1. Cellometer Auto 2000 User Manual

3.2. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance

3.3. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory

3.4. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

4. RESPONSIBILITIES

4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.

4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. DEFINTIONS

Term	Definition
CoA	Certificate of Analysis
SDS	Safety Data Sheets

6. REAGENTS, MATERIALS AND EQUIPMENT

6.1. Cellometer

6.2. Class II Biosafety Cabinet (BSC)

6.3. Pipettes and pipette tips

6.4. ViaStain AOPI Staining Solution (Nexcelom, Cat # CS1-0106-5mL)

6.5. Cellometer Check Validation Bead Solution (Nexcelom, Cat # CCBM-011-2mL)

6.6. Disposable Hemocytometer (Nexcelom, Cat # CP2-002)

6.7. Cavicide (Warehouse, Cat # 79300360)

6.8. Ster-ahol (VWR, Cat # 14003-358 or equivalent)

6.9. Wypalls Paper Towel (Warehouse, Cat # 79300335 or equivalent)

7. HEALTH AND SAFETY CONSIDERAIONS

7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.

7.2. Refer to the respective SDS when working with any chemicals.

7.3. Refer to “HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility” regarding waste disposal processes at the ATRF.

8. START UP

8.1. Turn on the Cellometer Auto 2000 by pressing the power button on the front of the unit.

8.2. Verify the instrument is connected to the network.

8.2.1. Click on the Windows Start icon.

8.2.2. Select “computer” and double click on the correct network drive to verify it is connected without any errors.

8.3. Prior to use, confirm the Quality Check was performed for the month per section 10. If it has not been performed, perform Quality Check and make entry on “HSL_EQ_006.01: Cellometer Auto 2000 Monthly Maintenance Form.”

9. COUNTING CELLS

9.1. Inside the BSC, mix cell pellet with cell culture media until mixture is homogenous.

9.2. A cell concentration range of 1.0×10^5 to 1.0×10^7 cells/mL can be analyzed by the Cellometer Auto 2000. A concentration of 1.0×10^6 is optimal. If undiluted cells are expected to be at a greater concentration than 1.0×10^6, dilute cells in media according to Table 1.

Table 1: Pre-Dilution Values

Dilution Factor	Volume of Cell Mixture (µl)	Volume of Media (µl)	Total Volume (µl)	Final Dilution Factor (after adding AOPI Stain)
2	50	50	100	**4**
3	30	60	90	**6**
4	25	75	100	**8**
5	20	80	100	**10**

9.3. Take 2 microcentrifuge tubes and add 20 µL of the cell mixture.

9.4. Add 20 µL of AOPI Stain to tube 1. Mix at least 5 times up and down using the same pipette used to add AOPI Stain.

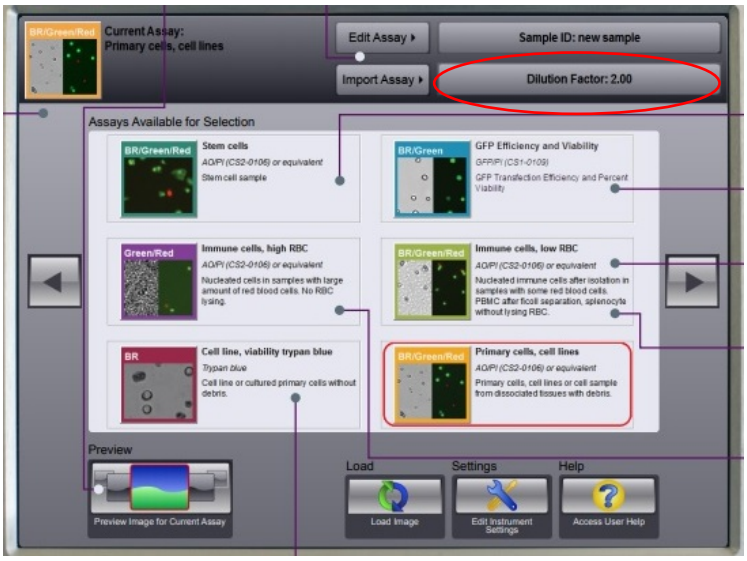
9.5. Using a new tip, add 20 µL of AOPI Stain to tube 2. Mix at least 5 times up and down using the same pipette used to add AOPI Stain.

9.6. Using a pipette set to 20 µL, mix tube 1 at least 3 times up and down then immediately add 20 µL of cell/AOPI Stain solution to side 1 of the hemocytometer chamber.

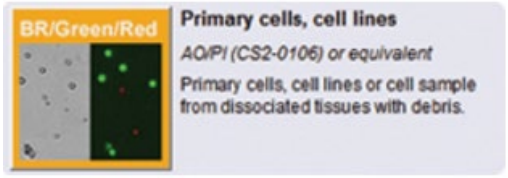
Note: If preferred, label slide chamber #1 and chamber #2 on the white margin of the chamber. Avoid touching the clear portion of the counting chamber.

9.7. Insert the loaded chamber into the Cellometer Auto 2000 sample slot and gently push the slide to the stop.

9.8. Make sure that the appropriate final dilution factor is listed on the Cellometer Auto 2000 home screen (See picture below). Without further dilution as is noted in Table 1, the dilution factor will be listed as “2” (20 uL cells + 20 µL AOPI Stain).



9.9. Click on the “Primary cells, cell lines” assay.



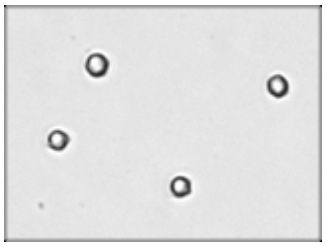
9.10. Click on “Preview Image for Current Assay.”

9.11. At the top left of the screen, using either the touch pad or the keyboard, enter analyst initials in “Enter User ID” field and use the following format for “Enter Sample ID” field:

Logbook Number / Lot Number_Date_Count #
(e.g., P0001_01Mar19_1)

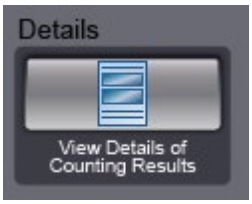
9.12. Hit “Save” at the bottom right hand side of the screen.

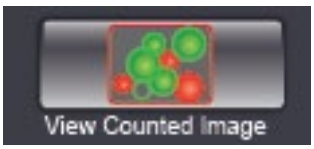
9.13. Adjust the focus if necessary using the coarse and fine adjustments on the left-hand side of the screen. Cells in focus have a bright center and a crisp edge.



9.14. Click the count button at the bottom of the screen.

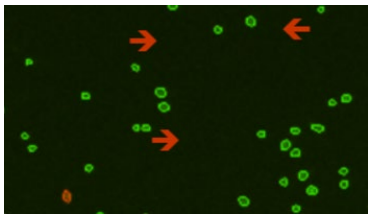
9.15. Click on the “View Details” button at the bottom left of the screen, then click on the “View Counted Image” button on the left-hand side of the screen.





9.16. Review the counted image. Nucleated cells with intact membranes stain fluorescent green and are counted as live, whereas nucleated cells with compromised membranes only stain fluorescent red and are counted as dead.

Note: Non-nucleated material, such as red blood cells, platelets and debris, do not fluoresce and are ignored by the Cellometer software.



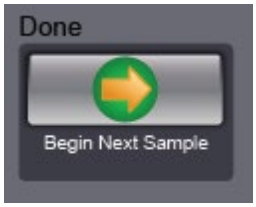
9.17. Click on the “Return” or “Back to Results” button. Cell count, concentration, mean cell diameter, and % viability are displayed. Record concentration and % viability on the associated form or in a Laboratory Notebook.

Note: Ensure the “live cell count” concentration is recorded for cell concentration (number of cells).

9.18. The Cellometer Auto 2000 is now ready to analyze the next sample.

9.19. Using a pipette set to 20 µL, mix tube 2 at least 3 times up and down then immediately add 20 µL of cell/AOPI Stain solution to side 2 of the hemocytometer chamber.

9.20. After inserting the imaging chamber loaded with sample 2, click on the “Next Sample” button at the bottom right of the screen.



9.21. When prompted, enter the Sample ID as described in step 9.11, then click “Count”.

9.22. Remove slide from counting chamber after use and discard.

9.23. Click on the “Return” or “Back to Results” button. Cell count, concentration, mean cell diameter, and % viability are displayed. Record results per step 9.17.

9.24. Turn instrument off after use and clean up any spills that may have occurred with Cavicide

10. CELLOMETER AUTO 2000 QUALITY CHECK

10.1. The Quality Check is performed every month when the instrument is in use, PRIOR to any cell counts being performed.

10.2. If the instrument is not actively being used, a comment is made on HSL_EQ_006.01. However, the quality check must be performed prior to performing any cell counts. For example, if the quality check was performed on 05Jan17, but the instrument was not used again until 25Mar17, then the quality check is performed PRIOR to counting cells on 25Mar17.

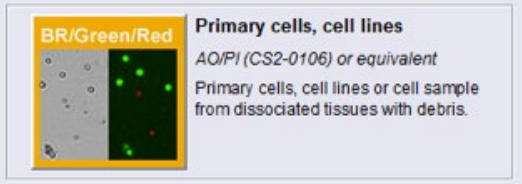
10.3. Obtain the CoA for the Reference Bead Solution, and record the Lot Number, Expiration Date, and ranges per the manufacturer for Concentration (Bead Solution Range) and Viability Specification on "HSL_EQ_006.02: Cellometer Auto 2000 Monthly Quality Check Form."

10.4. Vortex Reference Bead Solution for ten seconds prior to use.

10.5. Invert Reference Bead Solution ten times and immediately load 20 µL of mixed Reference Bead Solution into a new hemocytometer chamber.

10.6. Insert the loaded chamber into the Cellometer Auto 2000 sample slot and gently push the slide to the stop.

10.7. Select the Primary cell, cell line AO/PI assay.



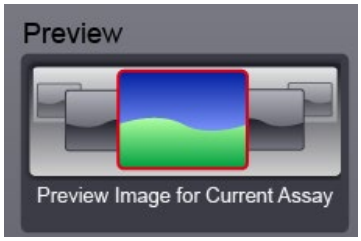
10.8. At the top left of the screen, using either the touch pad or the keyboard, enter analyst initials for "Enter User ID" field and use the following format for "Enter Sample ID" field:

Ref Bead_Date_Analyst Initials_Count #
(e.g., Ref Bead_01Mar19_ABC_1)

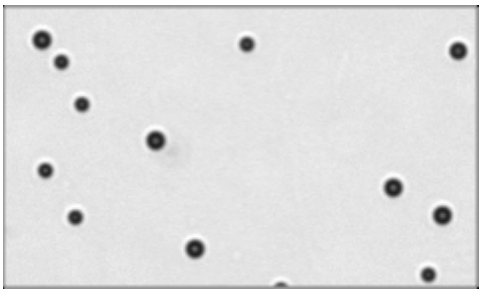
10.9. Hit "Save" at the bottom right hand side of the screen.

10.10. Set Dilution Factor to 1.

10.11. Click on “Preview Image for Current Assay.”

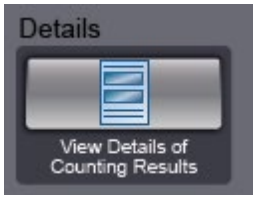


10.12. Adjust the focus if necessary using the coarse and fine adjustments on the left hand side of the screen until the best bead counting focus is achieved. The beads appear as dark circles with sharp edges.

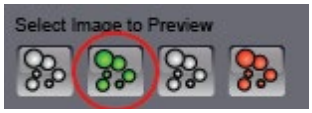


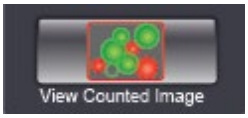
10.13. Click the “Count” button at the bottom of the screen.

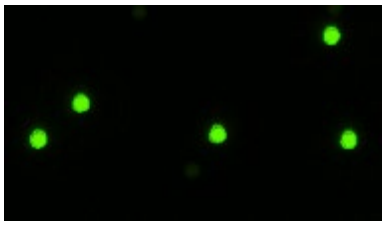
10.14. When counting is complete, click on “View Details” button at the bottom left of the screen.



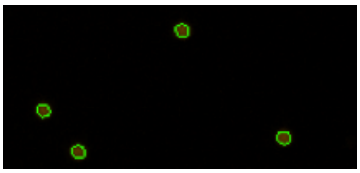
10.15. Select the green fluorescent image, then click on the “View Counted Image” button on the left-hand side of the screen. Enlarge the image by clicking the “Zoom In” button on the right-hand side of the screen. Confirm that all of the green fluorescent beads are circled in green.







10.16. Select the red fluorescent image. Confirm that all of the red fluorescent beads are circled in green.



10.17. Imaging and counting in the bright field, green fluorescent, and red fluorescent channels have now been confirmed.

10.18. Record the results under “measured concentration (Cells/mL)” and “measured viability (%)” on HSL_EQ_006.02. Remove slide from counting chamber.

10.19. For a passing Quality Check, the instrument results are within the manufacturer’s range.

10.20. If the Quality Check fails to be within the manufacturer’s range, repeat steps 10.3 to 10.18.

10.21. If the Quality Check fails to be within expected range a second time, obtain a new vial of Reference Bead Solution and repeat steps 10.3 to 10.18.

10.22. If the Quality Check fails a third time, immediately stop using the instrument and contact the Scientific Manager for next steps.

10.23. Turn instrument off after use and clean up any spills that may have occurred. Submit HSL_EQ_006.02 for review.

10.24. When passing results obtained, record maintenance on HSL_EQ_006.01.

11. ATTACHMENTS

11.1. Attachment 1: HSL_EQ_006.01: Cellometer Auto 2000 Monthly Maintenance Form

11.2. Attachment 2: HSL_EQ_006.02: Cellometer Auto 2000 Monthly Quality Check Form

Attachment 1: HSL_EQ_006.01: Cellometer Auto 2000 Monthly Maintenance Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: Cellometer Auto 2000 Monthly Maintenance Form			
Document ID: HSL_EQ_006.01		Version:	3.0
Associated SOP: HSL_EQ_006		Effective Date:	
Supersedes Version:	2.0	**Page 1 of 1**	

Maintenance Year:	
Equipment ID:	HSL_

Monthly Maintenance, HSL_EQ_006.02

Month	January	February	March	April	May	June
Recorded by/date:						
Reviewed by/date:						
Month	July	August	September	October	November	December
Recorded by/date:						
Reviewed by/date:						

Unscheduled Maintenance

Date	QE Number	Activity Performed	Recorded by/date	Reviewed by/date

QA Reviewed by/date: _____

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

Attachment 2: HSL_EQ_006.02: Cellometer Auto 2000 Monthly Quality Check Form

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

HPV Serology Laboratory
Standard Operating Procedure
Form

Form Title: Cellometer Auto 2000 Monthly Quality Check Form

Document ID: HSL_EQ_006.02		Version:	3.0
Associated SOP: HSL_EQ_006		Effective Date:	
Supersedes:	2.0	**Page 1 of 1**	

Equipment

Description	Identification Number
Cellometer Auto 2000	HSL_

Reagents

Description	Lot Number	Expiration Date
Reference Bead Solution		

Results

Reference Bead Solution Range (Beads/mL)	Measured Concentration (Cells/mL)	Result
		☐ Pass ☐ Fail
Reference Bead Solution Viability Specification (%)	**Measured Viability (%)**	**Result**
		☐ Pass ☐ Fail

Comments:
☐ First fail, repeat.
☐ Second fail, obtain new vial of Reference Bead Solution and repeat.
☐ Third fail, equipment placed out of service and Scientific Manager contacted.

☐ N/A

Performed by/date:	
Reviewed by/date:	
QA Reviewed by/date:	

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